

**REMARKS**

Favorable reconsideration, reexamination, and allowance of the present patent application are respectfully requested in view of the foregoing amendments and the following remarks. The foregoing amendments have full support in the specification and the original claims. No new matter is entered.

***Amendments***

Claims 1, 6, and 12-14 are amended. Claims 2-5 and 7-11 are cancelled. Claims 15-17 are new.

***Rejection under 35 U.S.C. § 103(a)***

In the Office Action, beginning at page 2, Claims 1, 6, 7 and 11-14 were rejected under 35 U.S.C. § 103(a), as reciting subject matters that allegedly are obvious, and therefore allegedly unpatentable, over the disclosure of Kojima *et al.* (hereinafter “Kojima”) in view of the disclosure of Calhoun *et al.* (hereinafter “Calhoun”), Ciccognani *et al.* (hereinafter “Ciccognani”), Spehr *et al.* (hereinafter “Spehr”), Kusomoto *et al.* (hereinafter “Kusomoto”), or Sone *et al.* (hereinafter “Sone”). Applicants respectfully request reconsideration of this rejection.

The claims recite a method for producing an L-amino acid by culturing an *E. coli* or coryneform bacterium in a medium and collecting the L-amino acid from the medium, wherein the bacterium is able to produce the L-amino acid and the bacterium has been modified to have enhanced activity of cytochrome bo-type oxidase. The bacterium can be further modified to be deficient in NDH-11 activity.

Kojima is cited for teaching methods of using bacteria for production of amino acids, and that *E. coli* and *Coryneform* bacteria are well-known for use in producing threonine, lysine, and phenylalanine. Kojima does not include any discussion of high energy efficiency pathways and low efficiency pathways, or the effects of altering these pathways. The Office Action cites to 5 secondary references as curing this deficiency, each in the alternative, and so each combination of Kojima with each of the secondary references will be addressed in turn.

First, Calhoun is cited for allegedly teaching, both explicitly and inherently, strains in which the bo enzyme is increased and NDH-II is decreased, and the effects on cell growth of these modifications. The Office Action states “Calhoun explicitly teaches that to increase growth efficiency, one would eliminate NDH-II or bd and increase bo.” However, there is no discussion or suggestion of the effect of these actions on L-amino acid production, and therefore, no logical reason to combine these teachings with those of Kojima. Calhoun only discusses the effect of these various changes in the cell on the cell growth, and does not mention or imply that there is any effect, either positive or negative, on L-amino acid production by the cell. In fact, Calhoun states “[t]he goal of this work is to determine the consequences of specific respiratory defects on the **growth** of *E. coli*” (emphasis added). However, one of ordinary skill in the art would know that increases in cell growth does not correlate to, or even imply, an improvement in L-amino acid production. That is, when the bacterial grow rate is high, new construction of cell components such as cell walls becomes necessary, and thus more carbon is consumed in the this cell component synthesis.

To the contrary, L-amino acids are preferably produced by fermentation under conditions when the **cell growth is suppressed** in order to prevent consumption of carbon sources for the formation of the cell components. Therefore, the yield of the target substances such as L-amino acids is decreased when the cell growth is increased. The person of ordinary skill in the art would know from reading Calhoun that making the changes taught by Calhoun, such as increasing activity of bo enzyme or decreasing NDH-II activity, will result in increased cell growth, but the person of ordinary skill in the art would also know that an increase in cell growth does not mean an increase L-amino acid production. In fact, the person of ordinary skill in the art would know that the L-amino acid production will actually be decreased as cell growth increases; and therefore, there is no logical reason or motivation to combine the teachings of Calhoun with those of Kojima and arrive at the claimed invention.

Ciccognani also cannot be combined with Kojima to arrive at the claimed invention. Ciccognani is cited for allegedly teaching methods of culturing *E. coli* in which an enzyme of the high-energy efficiency pathway is enhanced and an enzyme of the low-energy efficiency was deficient. However, Ciccognani does not teach or suggest

a deficiency of the NDH-II activity, and actually is a study of the binding properties of oxidases in *E. coli* and the effect of the depletion of copper on cell growth. It is shown that copper deficiency during growth results in depletion of copper from the oxidase. There is no discussion or suggestion of improved L-amino acid production. Therefore, similar to the combination of Calhoun and Kojima, there is no logical reason to combine Ciccognani and Kojima. This is because, as pointed out *supra*, there is no direct relationship between cell growth and L-amino acid production, and studies showing effects on cell growth under certain conditions such as that by Ciccognani bear no relationship to L-amino acid production. One of ordinary skill in the art would know that changes in cell growth does not correlate to, or even imply, an improvement in L-amino acid production. As Ciccognani does not teach, suggest, or even imply that any of the manipulations taught in their study relate in any way to L-amino acid production, and the person of ordinary skill in the art would know that changes in cell growth does not indicate an increase in L-amino acid production, there is no logical reason to combine the teachings of Ciccognani with those of Kojima.

Spehr also cannot be combined with Kojima to arrive at the claimed invention. Spehr is cited for allegedly teaching methods of culturing *E. coli* cells in which an enzyme of the high-energy pathway is enhanced. While this characterization is partially correct, the actual purpose of this study was to overexpress the entire Complex I in an attempt to understand more about its structure and interaction in the cell. Again, there is no discussion or suggestion of improved L-amino acid production by a cell in which this complex or any of the subunits is overexpressed. Therefore, similar to the combination of Calhoun and Kojima, and Ciccognani and Kojima, there is no logical reason to combine Spehr and Kojima. This is because Spehr fails to even mention L-amino acid production, increased cell growth, or any other effects of the overexpression of the Complex I. Spehr is merely trying to study the structure and function of this complex enzyme. By overexpressing the complex, Spehr was able to isolate all of the 13 subunits and cofactors. While interesting, this study and the results have no relevance to L-amino acid production. One of ordinary skill in the art would have no reason to combine the teachings of Spehr with those of Kojima as Spehr does not report as to the effects of overexpression of Complex I on any cellular function. The objective of Spehr is to

isolate a stable enzyme complex for further study. As Spehr does not teach, suggest, or even imply that any of the effects of overexpression of Complex I taught in their study relate in any way to L-amino acid production, there is no logical reason to combine the teachings of Spehr with those of Kojima.

Kusomoto also cannot be combined with Kojima to arrive at the claimed invention. Kusomoto is cited for allegedly teaching cells which are used in a method for producing amino acid, and that such cells can be altered to obtain improved amino acid production by altering the aerobic metabolism of the cell, specifically by deleting the low efficiency gene. The Office Action goes on to allege that Kusomoto directly link amino acid production with the growth yield and energy efficiency of the cell. Although Kusomoto does teach a cytochrome *bd type* oxidase gene; Kusomoto does not teach or suggest modifying a cell to enhance the activity of cytochrome *bo-type* oxidase nor the use of such a modified cell in the production of L-amino acids. Kusomoto states “in order to improve the growth of cells and synthesis of amino acids, it is important to understand the aerobic energy metabolism, more specifically the respiratory proton pumps in the bacterium” (page 390, right column, lines 4-7); however, one of ordinary skill in the art would not understand from this disclosure, either combined or not combined with Kojima, how to modify the respiratory proton pumps to achieve improved production of L-amino acids since this is a purely speculative statement. As Kusomoto does not teach, suggest, or even imply that enhancing activity of the *bo enzyme* may relate in any way to L-amino acid production, there is no logical reason to combine the teachings of Spehr with those of Kojima.

Sone also cannot be combined with Kojima to arrive at the claimed invention. Sone is not discussed in the Office Action except for the brief statement that Sone teaches strains with bo cytochrome oxidase activity and deficient cytochrome bd oxidase activity which have enhanced growth. However, Sone evaluated cytochrome bo-type oxidase and cytochrome bd-type oxidase, and reports that the growth of the strains with enhanced activity of these enzymes and a wild-type strain was on the order of the bo strain (cytochrome bo-type oxidase amplified strain) > wild type strain > bd strain (cytochrome bd-type oxidase amplified strain). However, this document only discloses the effect of cytochrome bo-type oxidase on oxygen consumption, proton transport and growth yield

(growth rate), and does not disclose the effect of cytochrome bo-type oxidase on L-amino acid production. Similar to the above points, improvement of oxygen consumption, proton transport and growth does not correlate to improvement of L-amino acid production. That is, when the bacterial grow rate is high, new construction of cell components such as cell walls is necessary, and thus more carbon is consumed in the synthesis of cell components. Therefore, one of ordinary skill in the art would understand that carbon flux into L-amino acid synthesis pathway may be reduced if cytochrome bo-type oxidase activity is enhanced, and so there is no reason or motivation based on the disclosure of Sone to combine these teachings with those of Kojima, in that there is no suggestion or motivation provided to enhance cytochrome bo-type oxidase activity for the production of L-amino acid. Furthermore, L-amino acids are preferably produced by fermentation under conditions when the cell growth is suppressed, in order to prevent that carbon sources are consumed for the formation of the cell components and the yield of the target substances such as L-amino acid is decreased thereby.

Finally, in regards to the final two secondary references, Kusomoto and Sone, the Office Action cites to the Office Action from the corresponding Japanese Application: “it is suggested to use enzyme genes relating to the electron transfer system in the respiratory chain, in order to improve the growth of cells and to produce the useful substance, such as, amino acid, with the better energy efficiency.” However, these sentences are the opinion of the Japanese Examiner, and neither Kusumoto nor Sone cited in the Japanese Office Action teach or suggest the use of enhanced cytochrome bo-type oxidase for producing L-amino acids.

In conclusion, there is no logical reason or motivation to combine any of the secondary references with Kojima, with the expectation to arrive at the claimed invention. None of the secondary references relate their respective cellular manipulations regarding bo-type oxidase with any indication or hint that such a manipulation will increase, or even effect, production of L-amino acids by the cell. As there is no logical reason to combine these various teachings with the primary teaching of Kojima, the claimed invention cannot be obvious over these references.

For at least the foregoing reasons, Applicants respectfully submit that the subject matters of Claims 1, 6, 7 and 11-14, each taken as a whole, would not have been obvious

to one of ordinary skill in the art at the time of Applicant's invention, are therefore not unpatentable under 35 U.S.C. § 103(a), and therefore respectfully requests withdrawal of the rejection thereof under 35 U.S.C. § 103(a).

***Conclusion***

For at least the foregoing reasons, Applicants respectfully submit that the present patent application is in condition for allowance. An early indication of the allowability of the present patent application is therefore respectfully solicited.

If Examiner Marvich believes that a telephone conference with the undersigned would expedite passage of the present patent application to issue, she is invited to call on the number below.

It is not believed that extensions of time are required, beyond those that may otherwise be provided for in accompanying documents. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. § 1.136(a), and the Commissioner is hereby authorized to charge fees necessitated by this paper, and to credit all refunds and overpayments, to our Deposit Account 50-2821.

Respectfully submitted,

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Date: March 4, 2010